



Research Article

Intrapopulation differences in biological traits and impacts in a highly invasive freshwater species

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Abstract

Individual variation assessments are essential to better understand population and community dynamics, as well ecosystem functioning. Although researchers have long recognized this aspect, only recently has evidence accumulated about the ecological importance of variation within species. The incorporation of individual variation provides an even more complete description of the effects a species may have on ecosystems and this detailed ecological knowledge can be especially important in the context of biological invasions. In this study, we used an invasion gradient of the signal crayfish *Pacifastacus leniusculus* in the Rabaçal River (NE Portugal) to assess possible intrapopulation differences in key biological traits and evaluate possible changes in ecological impacts. For this, we collected individuals from the core and front of an invasion gradient to characterize several traits such as abundance, size, sex-ratio, body condition, behaviour (i.e. boldness), and trophic niche. In addition, we performed two laboratory experiments to assess possible differences regarding the consumption of prey (gastropods), leaf mass loss, and nutrients release. Signal crayfish from the front of the invasion gradient have lower abundance, are larger, predominantly male, have better body condition in both sexes, exhibit increased boldness, and have higher $\delta^{15}\text{N}$ and lower $\delta^{13}\text{C}$ values. In addition, in experimental conditions, signal crayfish from the front of the invasion gradient consumed more gastropods and leaves and increased the concentration of nitrates and phosphates in the water. Overall, the signal crayfish has different biological traits and distinct ecological impacts along the invasion gradient in the studied river. Our study demonstrates the relevance of assessing biological traits and impacts of invasive species at the intrapopulation level.



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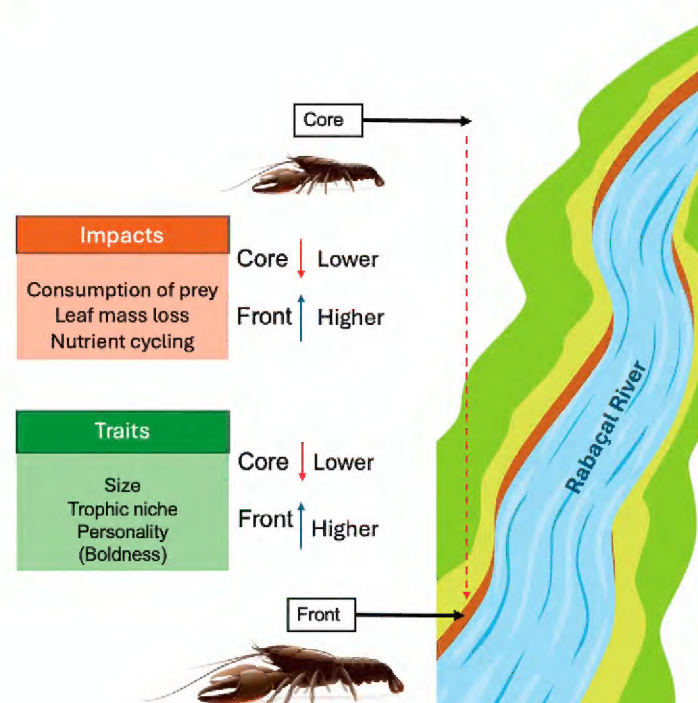
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Graphical abstract



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Key words: Biological traits, ecological impacts, invasive species, *Pacifastacus leniusculus*, signal crayfish

Introduction

Humans are key drivers of global environmental change (Dirzo et al. 2014) and anthropogenic activities have redistributed the world's biota and mediated species colonization of regions beyond their native range (Seebens et al. 2017; Pyšek et al. 2020). The consequences of these biological introductions are severe. Non-native species can disturb ecological communities and alter ecosystem functions, drive population declines and species extinctions, and continue to cost the global economy millions of euros every year given their detrimental negative impacts on several key ecosystem services (Ehrenfeld 2010; Strayer 2012; Simberloff et al. 2013; Diagne et al. 2021; Gallardo et al. 2024). Given the myriads of detrimental ecological, economic and social impacts attributed to non-native species and limited possibilities for total eradication, it is not surprising that their study is a prominent area of research (Pyšek et al. 2020). Although all ecosystems are susceptible to species introductions, freshwater ecosystems are especially vulnerable (Strayer 2010). This situation presents a challenge for the management and conservation of freshwater biodiversity because accelerated introduction rates may have detrimental consequences, including the erosion of biodiversity and as such, the disruption of key ecological processes and functions (Strayer 2010; Gallardo et al. 2016; Dudgeon 2019).

Understanding the species-level traits associated with invasiveness (defined in relation to arbitrary chosen quantitative metrics, which may encompass population abundance, geographical spread and ecological or socioeconomic impacts; Oficialdegui et al. 2024) has been a primary focus of biological invasions studies, with attributes such as high dispersal rates, high fecundity, and broad physiological tolerance among the key predictors of success (Pyšek et al. 2020). However, this may be an oversimplification (i.e. assessing the impacts, spread, or other topics at the species level) because a species can be very invasive in one region while simultaneously being almost innocuous a few kilometers away (Sousa et al. 2024). Moreover, a growing accumulation of evidence is showing that most of these impacts happen at the population level, not only due to the environmental context or biotic resistance of the invaded ecosystem (Blackburn et al. 2011), but also because intraspecific variation is increasingly recognized as an important driver of invasion dynamics (Biro and Stamps 2008; Haubrock et al. 2024).

In fact, environmental conditions can significantly influence the dynamics of biological invasions, including the establishment and spread of non-native freshwater species. Habitat structure, resource availability, and abiotic factors such as temperature, water quality, and flow regime can create heterogeneous landscapes that affect the success and impact of non-native populations (Lopez et al. 2022; Boon et al. 2023). Understanding how these environmental variables interact with biological traits is crucial for predicting invasion dynamics and impacts. It is likely that density-dependent processes, biotic factors (e.g. predation, competition and parasitism) and seasonal variability in abiotic factors interact to influence the life history traits observed at different stages of the invasion process (Bøhn et al. 2004). Given these idiosyncrasies, individual variation in ecological assessments is essential to understanding population and community dynamics, and ecosystem functioning (Bolnick et al. 2011; Violle et al. 2012; Des Roches et al. 2018; Raffard

et al. 2019). Although researchers have long recognized this aspect (e.g. Charles Darwin and Russel Wallace (Darwin and Wallace 1858) use these intraspecific variations as the central foundation of the theory of evolution), evidence of the ecological importance of variation within species has only recently accumulated (Post et al. 2008; Des Roches et al. 2018; Raffard et al. 2019). This last aspect may be related to variation in biological traits (e.g. size, sex, boldness, sociability, activity, aggression) and associated behavioural syndromes, i.e. correlations between these traits (Chapple et al. 2012). For example, personality, defined as individual differences in behaviours that are stable over time and context (Sih et al. 2004), can affect any stage of the invasion process (Juetten et al. 2014). This is because traits that maximize the probability of being introduced outside the native range may also promote successful establishment and spread within the recipient ecosystem (Myles-Gonzalez et al. 2015; but see Chapple et al. 2012 for discussion on possible exceptions). These biological traits are also likely to influence interactions with native species and ecosystems and thus may play an important role in determining the intensity of ecological impacts (Juetten et al. 2014). Despite the theoretical relevance, the fact is that very few studies have demonstrated how variation in biological traits may affect ecological impacts of a non-native species along an invasion gradient (Cote et al. 2010). However, biological traits, such as size, sex, body condition, and behaviour may act as important drivers on the spread dynamics of non-native species (Phillips et al. 2006) and may be responsible for distinct ecological impacts (Cote et al. 2010). For example, a study by Fryxell et al. (2015) showed that effects of a non-native freshwater fish (western mosquitofish *Gambusia affinis*) on phytoplankton and zooplankton abundance, as well as productivity, were influenced by variations in sex ratio and sexual dimorphism. Finally, these biological traits and impacts may vary not only spatially but also through time and this may differentially affect native communities over the years and may even have evolutionary consequences for native biodiversity (Bøhn et al. 2004; Mathers et al. 2016; Carvalho et al. 2022; Haubrock et al. 2024).

Therefore, it seems reasonable to assume that the incorporation of individual variation provides a more real and complete description of the population, community, and ecosystem being studied. Their downplay in empirical studies is probably related to the increased complexity of the analyses and due to additional workload or costs in laboratory and/or field sampling (Toscano et al. 2016; Sousa et al. 2024). Even so, a number of studies already demonstrate the importance of these intraspecific peculiarities in biological invasions, with the cane toad *Rhinella marina* invasion in Australia as the iconic textbook example (Shine, 2010). In this study, we use the recent invasion of the signal crayfish *Pacifastacus leniusculus* (Dana, 1852) in a Portuguese river to assess possible differences in biological traits and ecological impacts at the intrapopulation level. This species is native to North America and was first introduced in Europe, primarily in Scandinavia, in the 1960s for stocking purposes and replacement of the native crayfish species, which suffered great declines due to crayfish plague (Dunn 2012). *P. leniusculus* is characterized by high reproductive rates, fast-growing populations, wide environmental tolerance, high aggressiveness, and high dispersal abilities, making it a very successful invader in Europe (Dunn 2012). This species is also an ecosystem engineer, altering community structure, water quality, and nutrient dynamics (Carvalho et al. 2022). It is an omnivorous species and displays generalist and opportunistic feeding habits (Olsson et al. 2009). Ultimately, the signal crayfish may

play a crucial role in invaded ecosystems, holding a central position in food webs, where it functions both as predator and prey for many taxonomic groups, affecting different trophic levels and being able to have top-down and bottom-up cascading effects (Guan and Wiles 1998; Britton et al. 2017; Meira et al. 2019).

Given its high abundance and widespread distribution, the signal crayfish is responsible for several ecological and economic impacts on invaded ecosystems, but these impacts may be highly context-dependent even considering the same population. Therefore, the main aim of this study was to assess possible intrapopulation differences in key biological traits (abundance, size, sex-ratio, body condition, behaviour, and trophic niche) and ecological impacts (consumption of prey, leaf mass loss, and nutrients release) using the signal crayfish along an invasion gradient. Analogous to previous studies (e.g. Phillips et al. 2006; Brandner et al. 2013; Rebrina et al. 2015) we hypothesize that (1) there will be significant differences in abundance, size, body condition, and sex ratio between the core and the front of the invasion gradient; (2) signal crayfish individuals at the front of the invasion will exhibit bolder behaviour compared to those in the core area; and (3) these intrapopulation differences will result in varying per capita ecological impacts, with signal crayfish at the front of the invasion gradient consuming more gastropods and leaves, and contributing to higher nutrient concentrations.

Material and methods

Study area

This study was carried out in the Rabaçal River, at the Montesinho Natural Park and adjacent downstream areas (Fig. 1), located in NE Portugal. This river has a total length of 88 km, and its hydrological basin is subjected to a typically Mediterranean climate with an Atlantic influence, characterized by high seasonal variability in temperature and precipitation (Oliveira et al. 2012). This variability in precipitation (with annual rainfall ranging between 1000 and 1600 mm) is responsible for abrupt alterations in river flow, with maximum values being registered during winter/early spring and minimum values in late summer/early autumn (Sousa et al. 2018).

The low human density in the Montesinho Natural Park, as well as the land use mainly related to forest and subsistence agricultural activities (Nogueira et al. 2021a), makes this hydrological basin one of the least disturbed in Portugal, containing important habitats for several threatened freshwater species such as the pearl mussel, *Margaritifera margaritifera*, the dragonfly *Macromia splendens*, the Northern Iberian spined loach *Cobitis calderoni*, the Iberian desman *Galemys pyrenaicus*, among many others (Sousa et al. 2015, 2019, 2020). However, in the last two decades, the populations of these threatened species suffered a reduction in abundance due to the impacts of droughts, habitat loss and fragmentation, and the introduction of non-native species, such as the signal crayfish (Sousa et al. 2019, 2020; Nogueira et al. 2021b; Lopes-Lima et al. 2023). In Portugal, the signal crayfish was first detected in 1997 in the Maças River (NE of Portugal), the main tributary of the Sabor River (Bernardo et al. 2011; Anastácio et al. 2019). Over the following years, the species rapidly spread to almost the entire Sabor basin (Meira et al. 2019). In the Rabaçal River, the species was first detected in the core site (Fig. 1) in the summer of 2013 and from there spread exclusively in the downstream direction (Sousa et al. 2015; Carvalho et al. 2024). The exact date of introduction is unknown, and

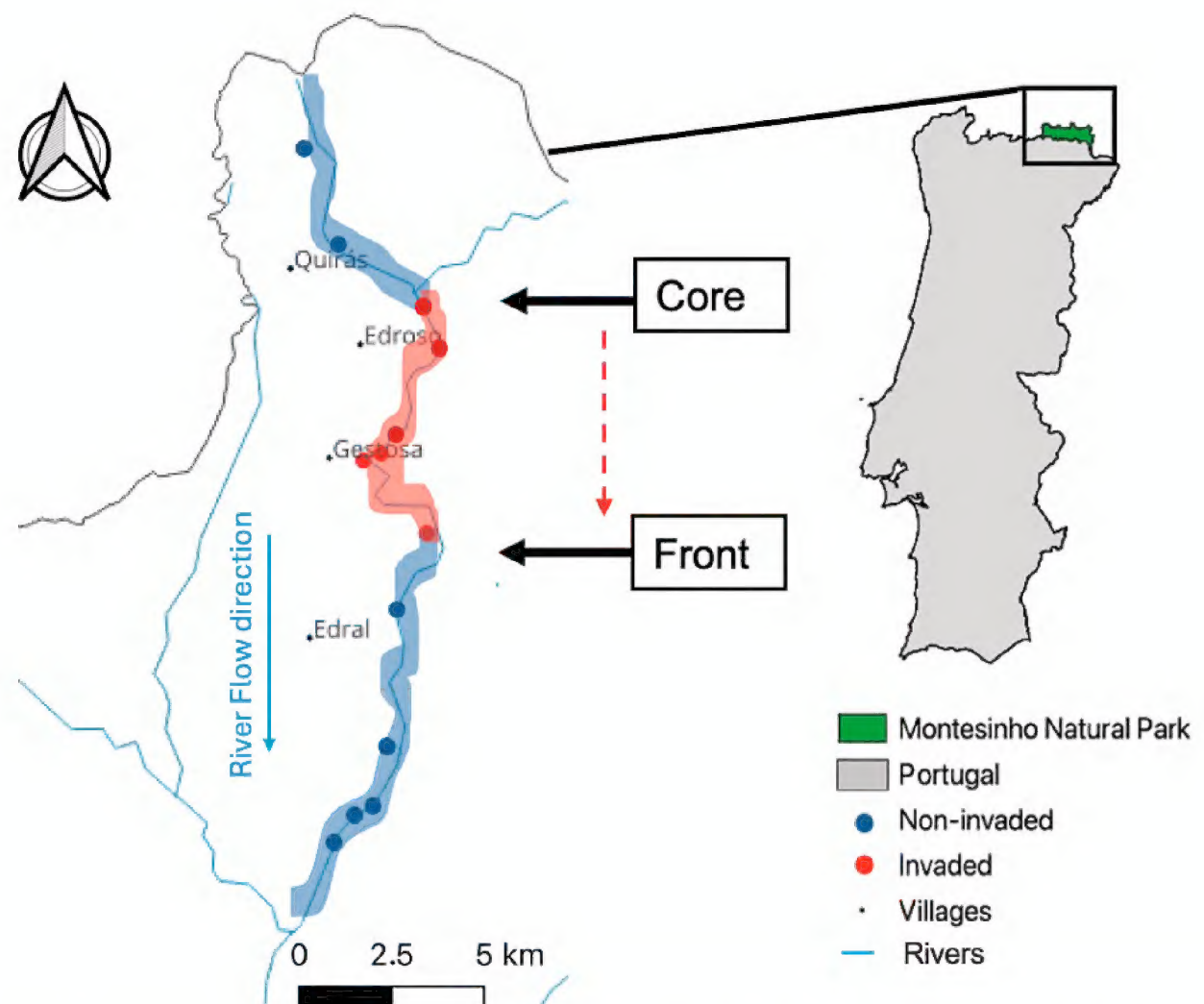


Figure 1. Map of the Rabaçal River with the location of both sites (core and front) sampled in this study. Marked dots in blue (non-invaded sites until 2023) and red (invaded sites) correspond to sampling sites monitored since 2017. The red dashed arrow indicates the spread direction of the signal crayfish.

it is believed that it was the result of one or more intentional introductions. Since 2017, this population has been monitored annually to assess their spread (Carvalho et al. 2024). This annual monitoring allows us to confidently establish the core (i.e. the original establishment site of the signal crayfish) and front (i.e. the leading edge of the signal crayfish invasion) of the invasion gradient (around 15 km distance between both sites) in the present study (Fig. 1). It should be noted, that besides the recent introduction of the signal crayfish, there are no records of any native or non-native crayfish species in the studied area (Sousa et al. 2019).

Environmental characterization

In both core and front sites (Fig. 1), in August 2023, we selected a river stretch of 100 m that comprised habitats including pools, runs, and riffles. In each site, temperature, conductivity, dissolved oxygen, and pH were measured using a YSI EXO 2 multi-parameter probe. Water samples were also collected to determine total suspended solids (TSS) as well as total organic suspended solids (OSS). For this, water volumes of 1 L were filtered using GFC filters, which were dried at 60 °C for 48 h and then heated in a muffle at 550 °C for 8 h. TSS and OSS were determined by weight difference following Zieritz et al. (2018). These measurements and water samples collections were made in the middle of the river near to the bottom at the end of the morning.

One sediment sample was also collected in each site, approximately 2 m from the left bank, using a cylinder with 10 cm of diameter. Granulometry and organic matter in the sediment of each site were determined following Sousa et al. (2007).

Both sites were also subjected to an in situ River Habitat Survey (RHS) to collect information related to the physical structure, including the complexity of the structure of the riparian vegetation, the type of aquatic vegetation, the characteristics of

the habitat and the type of artificial modification found in the channel and on the banks. For this, the standard length of 500 m of the river channel was used for data collection following Raven et al. (1998). All this collected information was used to obtain the Habitat Modification Score (HMS) and Habitat Quality Assessment (HQA) indices, which were important for ascertaining the degree of disturbance in both sampling sites (Raven et al. 1998).

Assessment of biological traits at the intrapopulation level

The study was conducted under permits (licence number 261/2023/CAPT) of the Portuguese governmental administration and conformed to international guidelines and national legislation regarding animal capture, manipulation, and experimentation for scientific purposes.

To test possible intrapopulation differences in biological traits between individuals from the core and the front of the invasion gradient in the Rabaçal River, crayfish abundance, total length, sex-ratio, body condition, behavior, and trophic niche were evaluated. Crayfish were captured by placing 8 and 12 funnel traps in the core and front sites (Fig. 1), respectively, for 24 h. These traps were specifically used for decapod crustaceans, they were all cylindrical (43 cm *d*, 22 cm *h*; 1.5 cm mesh) and were baited with dead fish (*Trachurus trachurus*). The captured individuals were counted, weighted, and measured from the rostrum tip to the telson rear edge (total length), and sex was determined following Sousa et al. (2013). The abundance of the signal crayfish per site was expressed as the total number of individuals per trap for 24 h (catch per unit of effort, hereafter ind. CPUE). In addition, the body condition of signal crayfish was assessed using individuals randomly collected in the field. A total of 80 (51 females and 29 males) and 38 (18 females and 20 males) individuals without visible injuries with a total length between 8 and 11 cm (in order to reduce possible bias; Rebrina et al. 2015), were measured in the core and front sites, respectively. For this, Fulton's condition factor was used following Rebrina et al. (2015), and it was calculated with the equation $K_c = 100 \cdot W/L^3$, where *W* is the total weight (g) of signal crayfish and *L* is the total length (cm).

Assessment of behavioral differences at the intrapopulation level

For the behavioral tests, a total of 40 captured signal crayfish in the field were immediately transferred to separate individual aquariums (to prevent any interaction between individuals) without food, located at the Polytechnic Institute of Bragança, where they remained for three days at a water temperature similar to field summer conditions (21 °C).

Two tests were conducted, each one using unique sets of 16 randomly selected individuals (8 females and 8 males) from both the core and the front of the invasion gradient (Fig. 1). The decision to include both males and females in our experiments was based on previous studies that have shown that sex can significantly influence crayfish behavior (Nakata and Goshima 2003; Gherardi and Cioni 2004). The experimental assessments were performed in an environmentally controlled room set at 21 °C in an aquarium with 46 × 63 × 32 cm (Suppl. material 1: fig. S1), and each individual was only used once. All individuals used in the behavioral tests were carefully examined to ensure they were intermoult and free of visible injuries in order to reduce possible bias (following Hudina et al. 2011).

The aim of the first and second tests were to evaluate boldness behavior associated with the presence of food and a novel environment, respectively. Average (\pm SD) of the signal crayfish from the core and front sites were 9.3 (\pm 0.6) cm and 9.6 (\pm 0.6) cm in the first test and 9.0 (\pm 0.7) cm and 8.9 (\pm 0.9) cm in the second test. Before testing, crayfish were allowed a 5-minute acclimatization period within a shelter (17 × 20 × 16 cm) placed inside the main aquarium, with the tested stimulus (food or the novel environment) already present in the main aquarium (following Brown et al. 2007; Suppl. material 1: fig. S1). After this period, the shelter lid was removed to initiate the tests. In the first test, a food source (cat food) positioned 30 cm away from the shelter at the opposite end of the aquarium was present. In the second test, a deep box open from above filled with stones and freshwater mussel shells was positioned 20 cm away from the shelter to simulate a novel environment (Suppl. material 1: fig. S1). In both tests, the time taken to exit the shelter was measured and the individual was considered to have left the shelter when its entire body was outside. New aquariums were used in each individual test. In both tests, the experiment was finished if the tested individual did not exit the shelter in 15 minutes.

Assessment of trophic niche at the intrapopulation level

To explore the trophic niche between the core and front of the invasion gradient, a total of 15 males and 15 females in the intermoult stage and without signs of injuries were randomly collected in both core and front sites of the invasion gradient (Fig. 1). The animals were measured (total length varied between 6.5 and 10.5 cm in the core and between 7.0 and 11.8 cm in the front) and immediately killed by freezing and were kept at -20 °C until the day of analysis. For stable isotope analysis, the animals were thawed; the abdominal muscle was extracted, dried in an oven for 24 hours at 40 °C, and then homogenised using a ceramic mortar. The elemental and isotopic composition analyses were conducted using a Thermo Scientific Flash 2000 model Organic Elemental Analyzer (EA), linked to a Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS) via ConFlo IV. The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of the samples and standards underwent normalization with certified reference materials for each element—IAEA-N-1, IAEA-N-2, and IAEA-NO3 for nitrogen, and USGS-24 and USGS-40 for carbon, achieving an analytical error margin of about 0.1‰. The isotopic composition of animal tissue samples was determined with precision, using an internal standard of sea bass, which was read after every 12 analyses to ensure analytical control. Corrections for the mass effect on the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were made according to a calibration curve derived from the delta values of N and C, which in turn were based on the peak amplitudes of various masses within the caffeine standard. Furthermore, the elemental composition was ascertained utilizing the K factor of *Chlorella*, with samples being analyzed in duplicates to maintain a coefficient of variation below 10%.

Assessment of ecological effects at the intrapopulation level

Individuals of *Physella acuta* (Drapanaurd, 1805) were used to assess consumption rates of crayfish individuals from the core and front of the invasion gradient. The individuals of *P. acuta* were captured using a hand net in the Fervença River.

We used this non-native species to reduce possible ethical problems associated with high prey mortality (see results) and because gastropod species are usually a preferred food item for signal crayfish (Rosewarne et al. 2016). The collected animals were rapidly transported to the laboratory and the shell length of individuals was measured from the apex to the tip of the last whorl with a caliper. The shell length varied between 6 and 10 mm, with an average (\pm SD) of 6.97 (\pm 1.12) mm. A total of 70 (35 individuals from the core and 35 from the front; Fig. 1) intermoult male signal crayfish captured in the Rabaçal River without signs of injuries were also used in this experiment. These signal crayfish captured in the field were immediately transferred to individual aquariums (to prevent any interaction between individuals), located at the Polytechnic Institute of Bragança, where they remained without food for three days at a water temperature similar to field conditions (21 °C). The selected individuals had an average length (\pm SD) of 8.2 (\pm 0.5) cm and 8.3 (\pm 0.5) cm in the core and front of the invasion gradient, respectively.

A total of 7 treatments with different abundances (2; 4; 8; 16; 32; 64; and 128 snails) of *P. acuta* were prepared. For each treatment, 5 replicates were used. Then, individuals of *P. acuta* were introduced into aerated aquariums (40 × 20 × 20 cm) containing 10 L of water but no sediment. Subsequently, one male signal crayfish individual was introduced into each aquarium. The signal crayfish individuals remained inside the aquariums for 24 h at a room temperature of 21 °C, and at the end of this period, the number of *P. acuta* individuals consumed were determined. The control treatment consisted of aquariums with *P. acuta* using the same abundances as described above but without crayfish.

Another laboratory experiment was conducted to assess possible differences in leaf mass loss following Carvalho et al. (2018, 2022). In summary, a total of 18 aquariums (40 × 20 × 20 cm) were prepared to replicate the most natural environment possible for the crayfish, so that their behavior was changed as little as possible. They were placed in a room with a temperature of 21 °C with an individual aeration system and, in each aquarium, stones were placed to provide a hiding refuge for the crayfish. Three different treatments were considered: six control aquariums with no crayfish; six representing the core of the invasion gradient; and the other six representing the front (Fig. 1) of the invasion gradient. A total of 10 L of water were added to each aquarium. After 24 h of aeration, the crayfish were placed in the aquariums and left 24 h without food. We only used male crayfish of approximately the same total length (average of 7.9 (\pm 0.5) cm and 8.0 (\pm 0.6) cm in the core and front treatments, respectively). Mesh bags with 4 g of dry alder leaves *Alnus lusitanica* were also prepared. Several other leaf-containing mesh bags were previously placed in the Rabaçal River for one week to be colonized by local microbiota (see Carvalho et al. 2018 and 2022 for detailed methodology); these were placed next to the prepared 4 g food bags for 24 h to serve as inoculum. After this, the leaves were removed from the 4 g food bags and placed in the aquariums for a period of 17 days (following Carvalho et al. 2018 and 2022). This time-period was more than sufficient (see results) to see a meaningful consumption of leaves. At the end of the experiment, the remaining leaves were collected with the aid of a 500 μ m sieve and then placed in an oven at 60 °C for 48 h. Finally, the leaves were weighed and the difference between the initial (4 g) and the final weight for each aquarium was calculated and then converted into a percentage of leaf mass loss. At the end of the

experiment, two water samples of 50 ml from each aquarium were collected to assess and compare the concentration of dissolved nutrients (ammonia, nitrite, nitrate, and phosphate) between the control, core and front treatments. For each nutrient, specific protocols were followed, using colorimetric methods (for details see Grasshoff et al. 1999). The readings were taken using a spectrophotometer HACH DR/2000 (HACH, Loveland, CO).

Data analysis

Differences in abundance between sites were analysed by non-parametric Kruskal-Wallis's tests, since the analysed datasets did not meet homoscedasticity and/or normality assumptions, even after several transformations. Differences in crayfish total body length and body condition between sites, sex and their interaction were compared by parametric two-way ANOVA. A chi-squared goodness-of-fit test was used to compare the observed numbers of males and females in the core and front sites with the numbers expected under a sex ratio of 1:1.

A two-way ANOVA was performed to investigate potential differences in stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) between sites, sex and their interaction. Following this, the isotopic niches of signal crayfish individuals from the core and front sites were analyzed using the SIBER method (Stable Isotope Bayesian Ellipses in R), as introduced by Jackson et al. (2011). This method entails the computation of ellipse areas, which were adjusted using SEAc (Standard Ellipse Area Correction) to account for sample size and other biases. The ellipses were then extended using Bayesian modelling to generate the SEAb (Bayesian Standard Ellipse Area). This approach allows for the comparison of isotopic niche sizes between groups. The application of SEAb served as a measure for comparing the relative sizes of ecological niches within different groups (Jackson et al. 2011). This was determined by the dimensions of the ellipses that were modelled using the isotope data, and by their predicted posterior distributions. When two groups have similar SEAb values, it suggests that the width of their isotopic niches is comparable, which indicates that their dietary ranges are likely similar (Jackson et al. 2011).

A two-way ANOVA was also performed to investigate potential differences in the time taken to exit the shelter for food or a new environment between sites, sex and their interaction. In addition, a Z-test was used to compare the proportions of individuals from front and core sites that exited the shelter within 15 minutes, to determine whether the observed difference was large enough to reject the null hypothesis that there was no difference between the two groups.

Two-way ANOVA was performed to evaluate gastropod consumption between individuals from the core and the front of the invasion gradient in the different abundance levels. One-way ANOVA was used to compare leaf mass loss and nutrients between treatments in the laboratory experiment.

When necessary, analyses were preceded by Shapiro-Wilk test to check the normality of the residuals and the Bartlett test to check for homoscedasticity, or normality was assumed if the number of observations satisfied the assumptions of the central limit theorem (Zar 1999). In the one and two-way ANOVAs we used Tukey "post hoc" tests to assess possible differences between pairs of groups. All the statistical analyses were carried out using the packages "SIBER" "ggplot2", "stats", "cowplot", "pgirmess", "phia" and "multcomp" (R Core Team 2021).

Results

Environmental characterization

The sampled sites are separated by 15 km and have very similar environmental conditions (Suppl. material 1: table S1). The studied river is subjected to a very low human disturbance and the RHS survey gives very similar results for both sites (Suppl. material 1: table S1). Consequently, we assume that the observed differences in biological traits and ecological impacts (see below) are not related to possible distinct environmental conditions between core and front sampling sites.

Biological traits at the intrapopulation level

Significant differences in abundance were found between the two sites (Kruskal-Wallis test, $\chi^2 = 8.15$, $p = 0.004$), with the core of the invasion gradient showing higher abundance (17.25 ± 13.38 ind. CPUE) when compared to the front (4.38 ± 4.47 ind. CPUE) (Fig. 2A).

Significant differences in the crayfish body length were found between sites (two-way ANOVA, $F = 132.32$, $p < 0.001$). However, there was no significant effect of sex ($F = 2.11$, $p = 0.15$) nor a significant interaction between site and sex ($F = 0.45$, $p = 0.50$). Signal crayfish individuals in the front of the invasion gradient were significantly larger than those in the core (Fig. 2B).

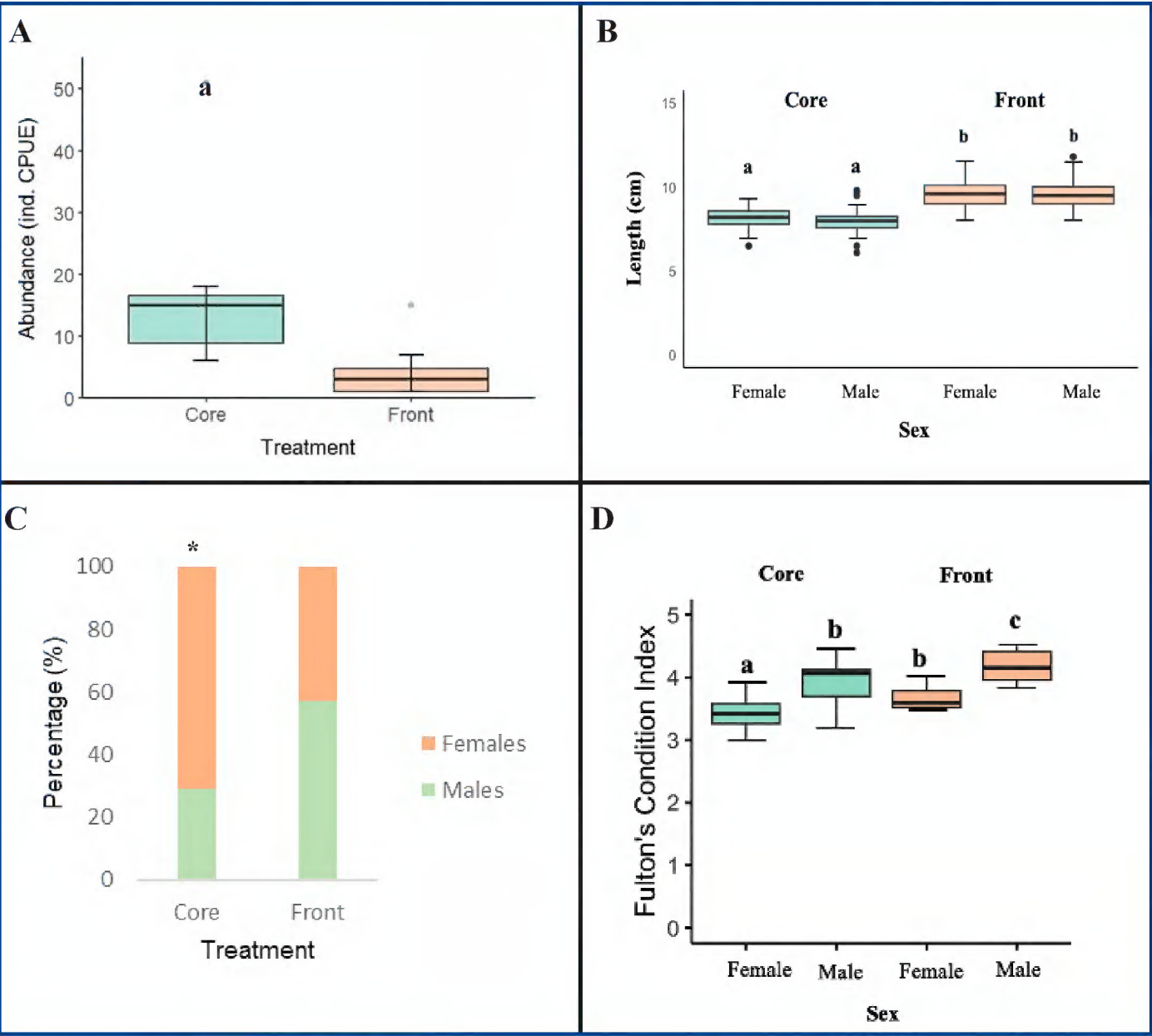


Figure 2. Abundance (A), body length (B), sex ratio (C), and Fulton's body condition index (D) for signal crayfish (*Pacifastacus leniusculus*) in the core and front of the invasion gradient. Boxplots show median values (central line), the interquartile range (box), and the non-outlier range (whiskers), and dots represent extreme values. Different lowercase letters or asterisk indicate significant differences.

There was a significant deviation from expected 1:1 sex ratio in the core of the invasion gradient ($\chi^2 = 24.38$, $p < 0.001$), which was dominated by females (71%). Males were slightly more numerous at the front of the invasion gradient (57%), but this was not significantly different from equal sex ratio ($\chi^2 = 0.714$, $p = 0.40$) (Fig. 2C).

Significant differences in the signal crayfish body condition using the Fulton's Condition Index were found between sites (two-way ANOVA, $F = 37.85$, $p < 0.001$), sexes ($F = 94.39$, $p < 0.001$), but no significant differences in the interaction between site and sex were detected ($F = 0.02$, $p = 0.89$), with individuals from the front of the invasion gradient and males presenting higher values (Fig. 2D).

Behavioral differences at the intrapopulation level

We observed no significant differences in the time for exiting the shelter with food between sites (two-way ANOVA, $F = 0.12$, $p\text{-value} = 0.731$), sexes (two-way ANOVA, $F = 0.01$, $df = 1$, $p\text{-value} = 0.96$) or the interaction between site and sex (two-way ANOVA, $F = 0.01$, $p\text{-value} = 0.95$). Similarly, we observed no significant differences in the time for exiting the shelter for new environment between sites (two-way ANOVA, $F = 0.742$, $p\text{-value} = 0.396$), sexes ($F = 2.34$, $p\text{-value} = 0.14$) or the interaction between site and sex ($F = 2.89$, $p\text{-value} = 0.10$).

However, there was a significant difference in the proportion of individuals that exited the shelter between the core and front sites (Z-test, $p = 0.032$). A higher proportion of individuals exited the shelter at the front site. Notably, 8 out of 16 individuals from the core group but only 2 out of 16 from the front group remained in the shelter.

Trophic niche at the intrapopulation level

Significant differences between individuals from the core and front of the invasion gradient was observed for $\delta^{15}\text{N}$ (two-way ANOVA, $F = 11.07$, $p = 0.002$) and $\delta^{13}\text{C}$ ($F = 10.51$, $p = 0.002$) values. In contrast, no significant differences were found between sex for either $\delta^{15}\text{N}$ ($F = 0.86$, $p = 0.36$) or $\delta^{13}\text{C}$ ($F = 1.78$, $p = 0.19$) values and interaction between site and sex for $\delta^{15}\text{N}$ ($F = 1.15$, $p = 0.29$) or $\delta^{13}\text{C}$ ($F = 0.53$, $p = 0.47$) values. The Tukey "post hoc" test further confirmed this, showing that the $\delta^{15}\text{N}$ mean value for the core was significantly lower than that for the front of the invasion gradient, with a shift of 0.58‰ (95% CI: -0.58 to -0.15 , $p = 0.001$). On the other hand, the $\delta^{13}\text{C}$ mean value for the core was significantly higher than that for the front of the invasion gradient, with a shift of -0.56‰ (95% CI: -0.92 to -0.21 , $p = 0.002$). The SIBER results highlight a separation between the isotopic niches at the invasion core and front (Fig. 3A). The isotopic niche overlap between males from the core and the front was notably small at 0.03‰^2 , equating to 2% of the area. For females, the overlap was even smaller, at 0.005‰^2 (0.3% of the area). When considering the animals of the same origin, the overlap between sexes was considerable at the front of the invasion gradient for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, at 0.68‰^2 , making up 54% of the cumulative area. At the core, a moderate male-female overlap was found, corresponding to 0.30‰^2 , or 36% of the area. The areas of the standard ellipses, which correspond to each group, varied, with ranges of 1.25‰^2 and 0.68‰^2 for females and males at the front of the invasion gradient, respectively, and of 0.50‰^2 and 0.66‰^2 for females and males at the core of the invasion gradient, respectively (Fig. 3B).

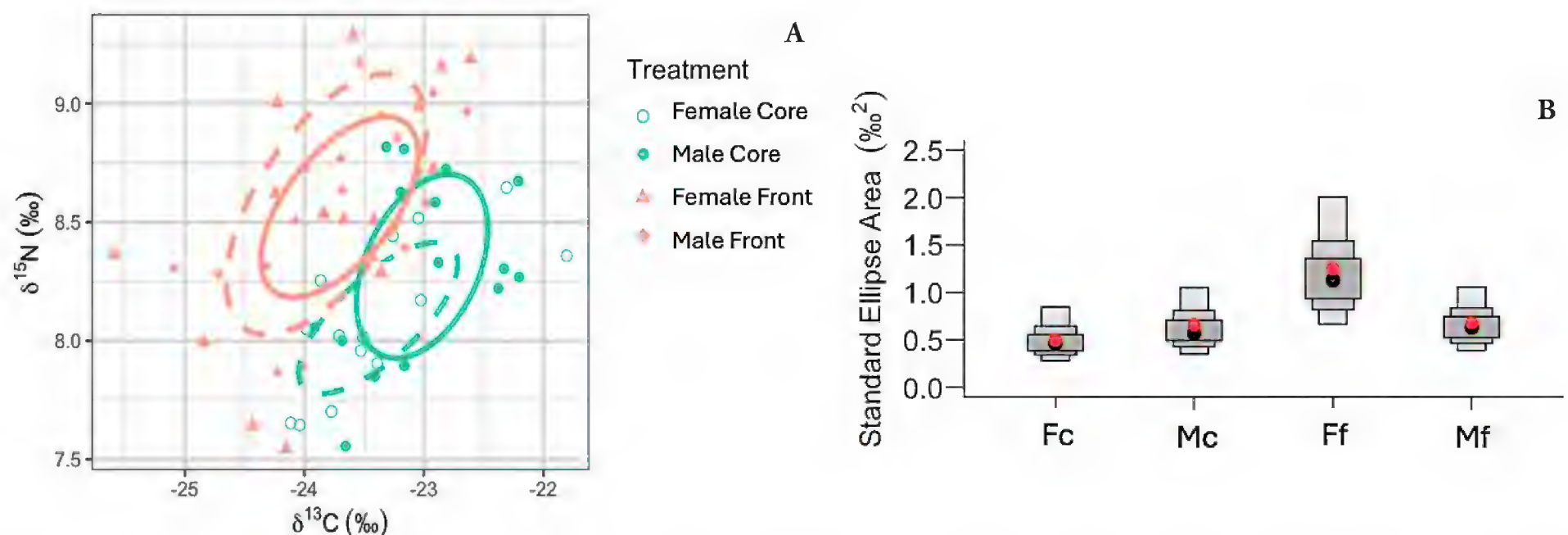


Figure 3. Isotopic niche (A) widths for adult signal crayfish muscle tissue from the core (blue) and front (red) individuals, with their sample-size corrected standard ellipses (SEAc). Standard ellipse areas (SEAb) (B) for the core: female (Fc) and male (Mc); and front: female (Ff) and male (Mf). Dashed and solid ellipses represent females and males from both the core and the front of the invasion gradient, respectively. The boxes represent the 95, 75, and 50% credible intervals, with the mode indicated by a black dot. The maximum likelihood estimate for the corresponding SEAc is indicated by a red dot.

Ecological impacts at the intrapopulation level

No gastropods were found dead in the control treatment. When analyzing *P. leniusculus* gastropod consumption, there was a significant interaction between abundance level of snails and the origin of the signal crayfish ($F = 8.51$, $p < 0.001$). In mesocosms with 2, 4, 8, 16, and 32 *P. acuta* individuals, practically all of them were consumed by the signal crayfish with no significant differences between individuals from the core and front of the invasion gradient (Fig. 4A, Suppl. material 1: table S2). However, for abundance levels of 64 and 128, significantly more gastropods were consumed by crayfish from the front of the invasion gradient ($\chi^2 = 18.15$, $p < 0.001$; $\chi^2 = 52.74$, $p < 0.001$, respectively) (Fig. 4A, Suppl. material 1: table S2).

Significant differences in the percentage of leaf mass loss were also detected (one-way ANOVA, $F = 70.30$, $p < 0.001$), with individuals from the front of the invasion gradient consuming more leaves (Fig. 4B). Both front and core groups were significantly different from the control treatment (Tukey "post hoc" test, $p < 0.001$ and $p < 0.01$, respectively), as well as between each other (Tukey "post hoc" test, $p < 0.001$).

Significant differences were detected for nitrate concentrations in the water after the leaf consumption experiment (one-way ANOVA, $F = 4.90$, $p = 0.023$) (Suppl. material 1: table S3), with the highest values in the treatments with signal crayfish from the front site (Fig. 5A). Significant differences were detected between front and control (Tukey "post hoc" test, $p = 0.048$) (Suppl. material 1: table S4) and between core and front treatments (Tukey "post hoc" test, $p = 0.034$) (Suppl. material 1: table S4). Significant differences were also detected for phosphate concentrations (One-way ANOVA, $F = 8.15$, $p = 0.004$) (Suppl. material 1: table S3), with the highest values in the front treatment (Fig. 5B). However, while significant differences were detected between front and control treatments (Tukey "post hoc" test, $p = 0.002$) (Suppl. material 1: table S4) the differences between the core and front treatments were not significant (Tukey "post hoc" test, $p = 0.069$) (Suppl. material 1: table S4). No significant differences between treatments were found for ammonia (One-way ANOVA, $F = 1.06$, $p = 0.375$) (Suppl. material 1: table S3, Fig. 5C) or for nitrite (One-way ANOVA, $F = 2.07$, $p = 0.172$) (Suppl. material 1: table S3, Fig. 5D) concentrations.

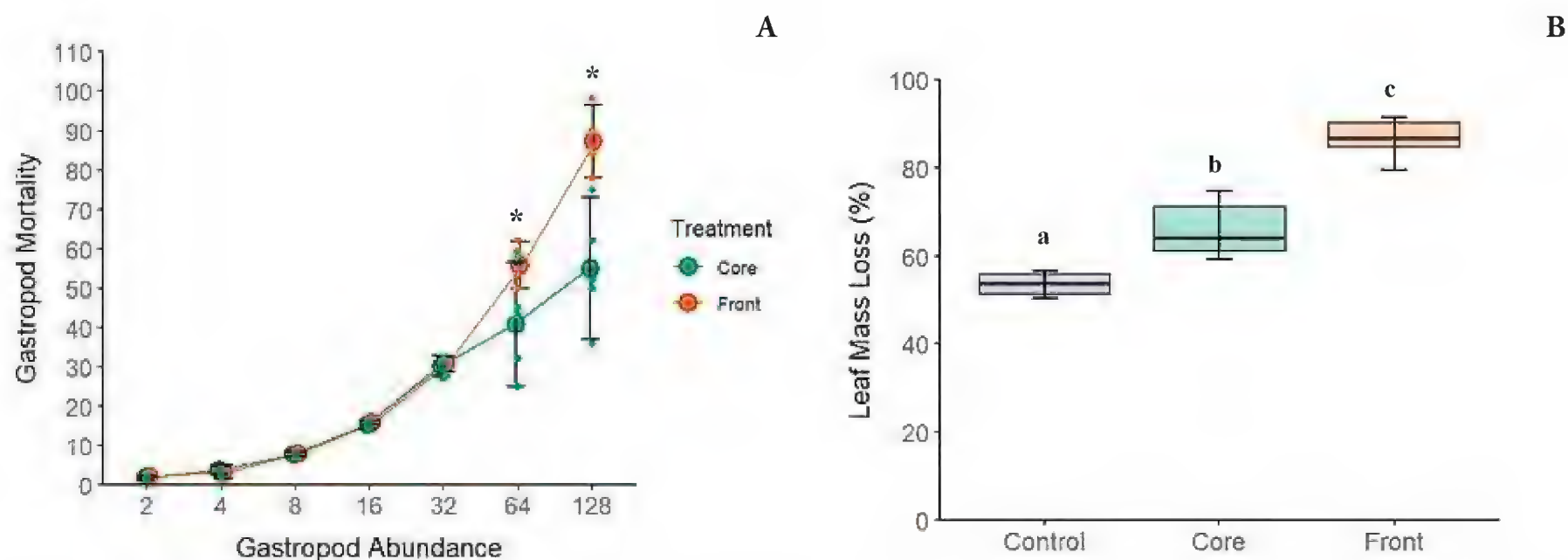


Figure 4. Number of gastropods consumed by the signal crayfish (*Pacifastacus leniusculus*) in each abundance level for core and front treatments (A) and leaf mass loss (%) for control, core and front treatments (B). The circles in A represent the mean morality values in each prey abundance level for the core (in red) and front (in blue) treatments; smaller red and blue dots represent the number of consumed gastropods in individual replicates. Boxplots in B show median values (central line), interquartile range (box), and range (whiskers). Different lowercase letters or asterisks indicate significant differences.

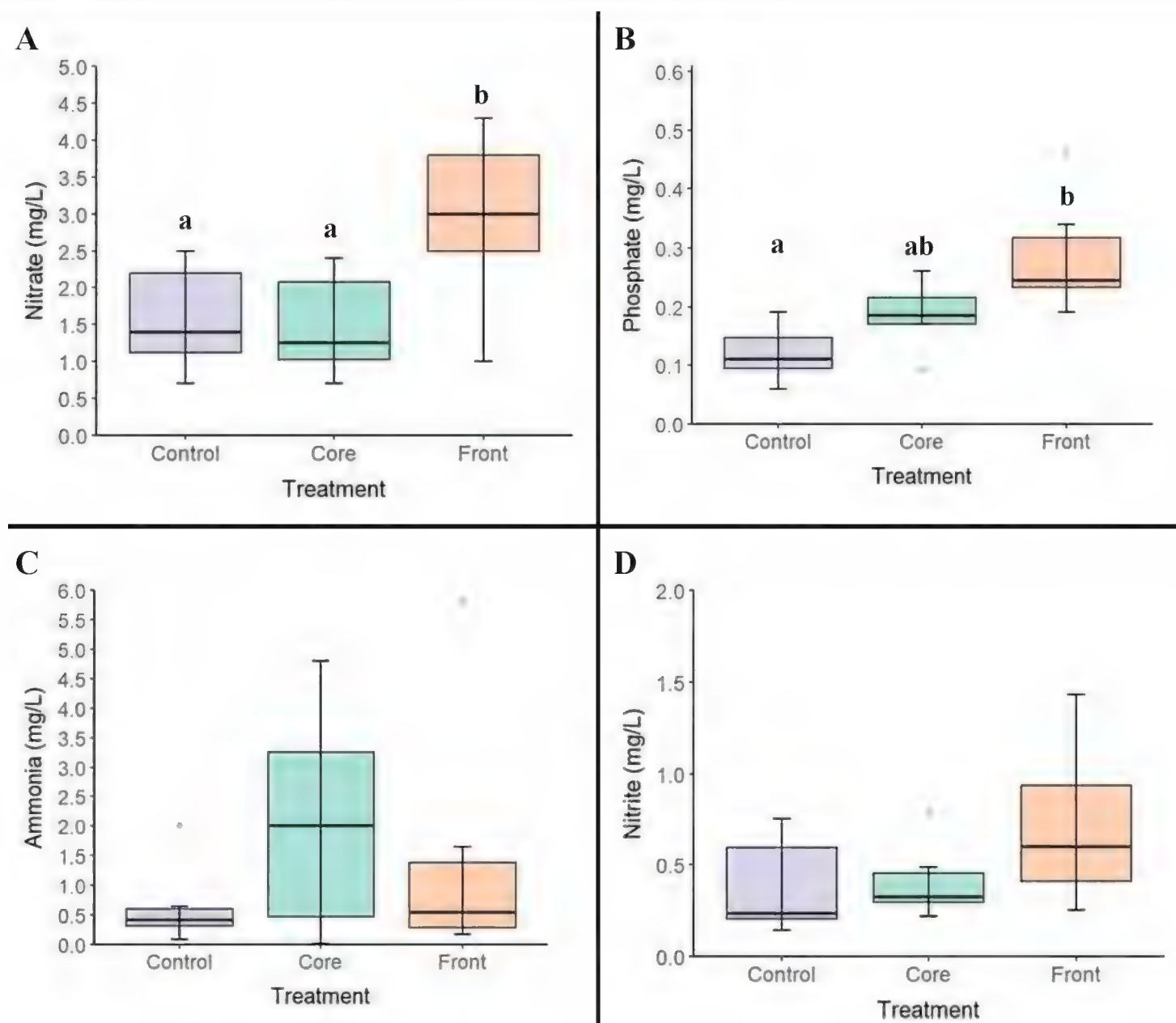


Figure 5. Concentrations (mg/L) of nitrate (A), phosphate (B), ammonia (C) and nitrite (D) in control mesocosms and in mesocosms with individuals from the core and front of the invasion gradient. Boxplots show median values (central line), interquartile range (box), range (whiskers), and dots represent extreme values. Different lowercase letters indicate significant differences.

Discussion

Natural populations consist of phenotypically diverse individuals who exhibit variation in their biological traits (Bolnick et al. 2011). However, these differences are usually neglected in biological invasion studies and most work is devoted to assessing ecological impacts at the species level (Haubrock et al. 2024). In this study,

we highlight that several biological traits and ecological impacts may vary along an invasion gradient with clear differences established at the intrapopulation level. In the particular case of the signal crayfish in the Rabaçal River, we clearly showed that abundance was higher in the core of the invasion gradient and females dominated there; while larger individuals with a higher body condition were detected at the front of the invasion gradient where slightly more males than females were captured. Regarding behaviour, we demonstrated that the animals in the front were bolder. In the same vein, individuals from the core and front of the invasion gradient occupied clearly distinct trophic niches, with individuals from the front having higher $\delta^{15}\text{N}$ and lower $\delta^{13}\text{C}$ values. These differences in biological traits are consistent with distinct ecological impacts as assessed by aquarium experiments, with higher consumption of gastropods, leaf mass loss, and release of nutrients by individuals from the front of the invasion gradient.

Biological traits at the intrapopulation level

Signal crayfish abundance was lower in the front compared to the core of the invasion gradient and individuals were larger at the front of the invasion. On a simplistic level, it is obvious that in the newly invaded sites, there will be lower abundance, given that signal crayfish individuals are still arriving at the front of the invasion. Similar differences in abundance and size were also reported in many other animal invasions (see for example Bøhn et al. 2004; Wolf and Weissing 2012; Brandner et al. 2013; Raffard et al. 2022), including on non-native crayfish populations (Hudina et al. 2012; Nawa et al. 2024). Differences in size could be explained by the fact that larger individuals can move faster and thus are the ones who lead the invasion (Hudina et al. 2012). Similar findings regarding the influence of size in dispersal were recorded in a study with cane toads, *Bufo marinus*, in Australia, where toads with longer legs were the faster ones and the first to reach new sites and so leading the invasion front (Phillips et al. 2006). Another factor possibly contributing to the differences in size between our study sites could be the lower level of recruitment in the front of the invasion gradient given the low abundance of crayfish in this site. Finally, individuals could be growing faster due to density-dependent effects or due to trophic niche shift to more nutritious food source in the front of the invasion gradient (Hudina et al. 2012; Brandner et al. 2013). In this study, we were not able to determine the main mechanism explaining our results regarding the size of the animals and all these hypotheses should be tested in future works.

Differences in sex ratio were also detected between the core and front of the invasion gradient. At the core, the percentage of females was significantly higher than males but at the front, the percentage of males was slightly higher than females. Higher male percentage in the front of the invasion were also reported for round goby (*Neogobius melanostomus*) in Canada (Gutowsky and Fox 2011), or for other signal crayfish populations in Europe (Capurro et al. 2007; Wutz and Geist 2013). Those results, according to the authors, were related to recent, not fully established invasive populations. The sex ratio is possibly important since it may interfere with some ecological processes. For example, female-biased populations of western mosquitofish *Gambusia affinis*, in which males are substantially smaller than females, are able to induce stronger pelagic trophic cascades than male-biased populations, causing larger impacts on communities and ecosystems (Fryxell et al. 2015). A study on the invasive freshwater crayfish *Procambarus clarkii* showed that females

have larger home ranges (Barbaresi et al. 2004). The higher percentage of females in the core of the invasion might also be related to a higher number of encounters and fights among more aggressive males as a result of higher crayfish abundance and a consequent decrease in available space and resources (Sousa et al. 2013). This might result in the death of some male individuals or the exclusion of the weakest in sites with higher abundance. Alternatively, it can be related to bolder male crayfish individuals that will be more prone to explore downstream areas (Raffard et al. 2022).

We also observed that signal crayfish individuals in the front of the invasion gradient had a higher body condition when compared with the individuals in the core. This may be related to a lower competition for resources at the front, which allows these individuals to have easier access to a higher amount or more nutritious food (see below further discussion on trophic niche) and to shelters, and in doing so they possibly increase their body condition (Brandner et al. 2013; Rebrina et al. 2015). Although not studied, another explanation for these results may be related to a lower level of predation (e.g. by river otter *Lutra lutra* and brown trout *Salmo trutta*) and/or parasitism in the front when compared to the core. For example, studies with cane toads in Australia showed that pathogens and parasites lag about 2 years behind the front of toad invasion, due to stochastic events that lead to local extinctions or transmission failure of the pathogens/parasites in the front of the invasion (Phillips et al. 2010; Brown et al. 2013). However, this last aspect was not assessed here and future studies should explore this topic. Furthermore, and as described in another study with signal crayfish populations (Rebrina et al. 2015), the condition index is sex-dependent.

Behavioural differences at the intrapopulation level

Several organisms rapidly change their behavioural traits to expand their distribution area and be able to make decisions that involve risks (Yagound et al. 2022) due to the new challenges imposed by the environment and the pressures arising from their expansion (Biro and Stamps 2008). What we observe in this study is a behaviour change in geographical terms, with the animals at the front being bolder and more willing to take risks than those at the core of the invasion gradient (Gruber et al. 2018). Similar results were obtained by Groen et al. (2012) and Myles-Gonzalez et al. (2015) for the round goby in non-native areas where the individuals at the front took more risks and moved faster. Several studies have also concluded that individuals at the front of the invasion gradient tend to be more exploratory than those at the core (Atwell et al. 2012; Liebl and Martin 2012, 2014).

Trophic niche at the intrapopulation level

In addition to differences in several biological traits as discussed above, we also observed higher $\delta^{15}\text{N}$ and lower $\delta^{13}\text{C}$ values in signal crayfish individuals from the front compared to the core of the invasion gradient. These differences in $\delta^{13}\text{C}$ suggest a greater reliance on submerged vegetation, periphyton, and detritus as important dietary sources in the core, possibly reflecting the increased competition for resources in this site (Ercoli et al. 2021). The elevated $\delta^{15}\text{N}$ values observed in signal crayfish at the invasion front suggest a dietary shift towards a reliance on a higher proportion of consumers such as macroinvertebrates. This shift in diet is likely a response to the specific ecological conditions at the invasion front, where competition for

resources is presumably less intense compared to the core of the invasion gradient, where crayfish abundance is significantly higher (Brandner et al. 2013; Hudina et al. 2017). Although direct diet sources were not assessed in this study, it is plausible that crayfish in core areas consume more submerged vegetation and detritus. Notably, female crayfish in the core of the invasion gradient exhibited a smaller niche area, suggesting that the heightened competition for limited food resources compels them to consume a lower range of dietary items at lower trophic levels. In contrast, signal crayfish at the front of the invasion gradient may have greater access to invertebrates and other animal prey (possibly including dead animals), which are readily available in the initial phase following their arrival. The relatively low abundance of crayfish at the front of the invasion gradient likely reduces competition, facilitating this reliance on higher trophic levels (Bubb et al. 2004; Brandner et al. 2013; Greenhalgh et al. 2022). Increased boldness among crayfish at the front of the invasion gradient could further enhance their ability to exploit these available resources, thereby providing a competitive advantage in newly invaded areas. However, this situation remained untested, and future studies are necessary to clarify this situation.

Ecological impacts at the intrapopulation level

This study also highlighted that the ecological impacts may vary along the invasion gradient with clear differences established at the intrapopulation level. In the particular case of the signal crayfish in the Rabaçal River, the consumption of gastropods, leaf mass loss, and the concentration of nitrates and phosphates were significantly higher in the aquariums with crayfish individuals from the front of the invasion gradient.

Dispersal is one fundamental ecological process where these intraspecific variations, particularly the personality traits (e.g. activity, boldness, aggression, and exploration) may play a crucial role (Daniels and Kemp 2022). Personality traits are often correlated with dispersal and this correlation is also known as a dispersal syndrome (Biro and Stamps 2008; Galib et al. 2022; Raffard et al. 2022). These dispersal syndromes have been reported in a wide range of taxa such as great tits, *Parus major* (Dingemanse et al. 2003); common lizards, *Lacerta vivipara* (Cote and Clobert 2007); North American red squirrels, *Tamiasciurus hudsonicus* (Cooper et al. 2017); mud crabs, *Panopeus herbstii* (Belgrad and Griffen 2018), among others. Activity, boldness, and exploration traits are often linked with better fitness, and individuals with these traits enhanced are expected to disperse further (Juette et al. 2014; Galib et al. 2022), and in so doing they lead the invasion front. Some of these personality traits have previously been observed in the signal crayfish (Galib et al. 2022), and our results also showed that signal crayfish individuals in the front of the invasion gradient were bolder than individuals in the core.

Since individuals with this type of behaviour are more prone to disperse, this can be associated with higher energy expenditure, which was counterbalanced by higher consumption rates (Raffard et al. 2022). This could be the main reason explaining our results regarding gastropod consumption, where we observed no differences between core and front individuals in the low prey abundance treatments but higher consumption of prey in the high-abundance treatments by the crayfish from the front of the invasion gradient. Similar results were also obtained in the experiment with leaf mass loss. This suggests a higher voracity of crayfish from the front of the invasion gradient because of their higher metabolic demands (Raffard et al. 2022).

Our study also made it possible to verify that the signal crayfish influences key ecosystem processes, as is the case of leaf litter processing and nutrient cycling. We observed that signal crayfish had an effect in leaf litter processing. Similar results have been reported for the red swamp crayfish, *Procambarus clarkii* (Carvalho et al. 2016 and 2018). Since leaf litter processing is driven by a group of animals that control the flux of carbon and energy in aquatic food webs (Carvalho et al. 2018), fundamental processes like nutrient cycling and organic matter turnover (Jackson et al. 2014), could be affected by the invasion of signal crayfish, even at the intrapopulation level. In addition, higher nutrient concentrations were also found in the front treatment, in line with a study by Villéger et al. (2012) where *Salmo trutta* individuals that were capable of dispersing further were the ones that displayed a higher excretion rate of nitrogen and phosphorus.

Overall, we found a higher consumption of gastropods and leaf litter, and a higher concentration of nutrients, namely nitrates and phosphates, in the experiments with crayfish from the front of the invasion gradient, where bigger and bolder individuals, supposedly with higher metabolic rates, were present. Therefore, these intrapopulation interactions can cascade to the population and community levels due to a different consumption of prey and/or leaf litter and being responsible for nutrient-mediated effects, modifying nutrient cycling at the ecosystem level. However, and as clearly demonstrated here, although the per capita consumption of prey and excretion rates were significantly higher at the front of the invasion gradient it should be noted that the abundance in the two sites is quite different, being much higher in the core of the invasion gradient.

The results of this study clearly demonstrated significant differences at the intrapopulation level, but some questions still remain unanswered and open the door for future studies. For example, it would be interesting to assess the metabolic rates in individuals from the core and front of the invasion gradient as the possible key mechanism explaining the different consumption rates and nutrient concentrations reported here. In the same vein, biological traits can also be related to the expression of specific genes (Yagound et al. 2022) and future studies may include a comparison of genetic variation and gene expression between individuals from the core and front of the invasion gradient to test whether the observed changes are due to selection of certain genotypes favouring dispersal or whether the observed differences are due to phenotypic plasticity (and, possibly, differential gene expression) (Cox 2013). Finally, and because animals in the front of the invasion gradient may have left their enemies behind, it would be interesting to assess the level of predation and parasitism in the core and front of the invasion gradient and their possible contribution to the distinct ecological impacts.

Conclusion

In this study, we demonstrated distinct biological traits and ecological impacts of signal crayfish along an invasion gradient. Currently, theoretical and empirical studies in biological invasions focus mainly on the species level (e.g. compilation of deny lists), especially those that have high ecological and economic impacts. However, as shown here, biological traits and ecological impacts can be very different at the intrapopulation level. Recognizing that individual variation has important ecological and evolutionary consequences, the assessment of biological traits at the intrapopulation level may help better predict the success of dispersal and the

ecological impacts generated by non-native species, with eventual pay-offs in the implementation of meaningful management actions. In the particular case of this study, it could be interesting to implement targeted removal and population control in the front of the invasion gradient to reduce density-dependent processes and remove disperser-adapted phenotypes from the gene pool.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: RS. Formal analysis: HA; DG; ABN; AT; JD; RS. Funding acquisition: RS. Investigation: HA; DG; ABN; AT; JD; RS. Methodology: RS. Resources: RS. Supervision: RS. Writing – original draft: HA; DG; RS.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Additional information

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Data type: docx

Explanation note: **fig. S1.** Experimental setup for testing signal crayfish behavior. (a) The shelter and food were placed at opposite ends of the aquarium. (b) The shelter with a new environment consisting of stones and shells of freshwater mussels. **table S1.** Environmental characterization of Core and Front sampling sites. HMS - Habitat Modification Score; HQA - Habitat Quality Assessment. **table S2.** Summary of Chi-square (χ^2) tests for each abundance level of gastropods. The asterisk show a significant difference in abundance levels between the core and front treatments. **table S3.** Summary of One-way ANOVA tests for nutrients concentration. The asterisks indicate significant differences in nutrients concentration between the core, front and control treatments. **table S4.** Summary of Tukey post hoc tests for the concentration of nitrate and phosphate. The asterisks indicate a significant difference in concentration of nitrate and phosphate between the core, front and control treatments.

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